

In Vivo Determination of the Complex Elastic Moduli of Cetacean Head Tissue

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LONG-TERM GOALS

The overall goal of this project is to develop and demonstrate a system for non-invasive *in vivo* measurement of the complex elastic moduli (stiffnesses and loss factors) of cetacean head tissues. This system is ultimately intended to provide a portable diagnostic capability for use in stranded animal assessments.

OBJECTIVES

The primary technical objective is to remotely generate and detect mid-frequency elastic waves within the body of a living cetacean and to use the measured propagation parameters of these waves to obtain the complex elastic moduli by inversion. A further technical objective is to extract tissue moduli in this manner intracranially. This objective carries considerably more technical risk since both the wave-generating ultrasound and the probe ultrasound will be attenuated, distorted and scattered by the passage through the skull. The final objective is to develop a prototype portable version of the technology and use it to perform examinations of stranded animals. Data collected with this system is envisioned to serve two purposes: 1) provide basic knowledge of in-vivo elastic properties, which is non-existent for marine mammals, and 2) provide a potential basis for non-invasive diagnostics of tissue pathologies, both naturally occurring or otherwise induced.

APPROACH

The foundation of the work is the capability to remotely generate elastic waves in soft tissues and observe their propagation with an ultrasound-based non-invasive system. The general approach for generation, reception and interpretation of the tissue wave fields is based on a new medical imaging technology called radiation force elastography¹. These techniques, which have been demonstrated to some extent on human soft tissues, cannot be directly translated to use on cetacean head tissues due to the need to propagate through much thicker tissues and through skull bone, all the while keeping

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within safety limitations for ultrasound exposureⁱⁱ. The current focus of the *in-vivo* program is to overcome these challenges through novel redesign of the concepts for both elastic wave generation and observation.

Two embodiments of the wave generation system, involving different methods of shaping the forcing field, are being investigated. The first method involves generation of a line-like force and measurement of the waves which diverge from it. This requires a receiver or force generator whose focal volume is moved mechanically. A second embodiment is being studied wherein a ring-shaped forcing volume is formed by the ultrasonic source. As the ring radius is changed (through a change in the ultrasonic carrier frequency, for example), a fixed receiver measures the change in phase of the waves converging to the center of the ring. This approach has several potential benefits, the sum of which are expected to directly translate to an improvement in the safety of the system relative to ultrasound exposure levels in living mammalian tissues.

The particle displacements resulting from the remotely generated elastic waves can be detected remotely using a modified version of an ultrasonic Doppler vibration measurement system called NIVMS developed at Georgia Techⁱⁱⁱ. Algorithms are being developed to enable the magnitude and phase of vibration to be determined, as well as the range (tissue depth) along the ultrasonic beam at which the vibration is being measured. By measuring the amplitude and arrival time of the shear wave at two different points (or from waves arriving from two different drive locations, as with the ring force excitation) the propagation speed and loss can be determined.

Elastic waves will be both remotely and directly generated in tissue phantoms and measured both remotely and directly to validate the measurement technique. The elastic properties of tissue phantoms will be obtained from remotely generated and measured data and compared with directly measured and tabulated material values. The noninvasive technique will be repeated for tissue phantoms enclosed in a simulated or hydrated real cetacean skull, and with harvested tissue samples. In-vivo testing will be conducted on Navy dolphins. Ultrasound parameters (peak negative pressure, time averaged intensity) will be consistent with limits established as safe for humans, and ultrasound frequencies will be kept high enough to be far above the highest frequency that is audible to the animal.

WORK COMPLETED

1. Ultrasonic Vibrometer Development The prototype range-resolving ultrasonic vibrometer was further refined and demonstrated through a series of tests with a tissue phantom which mimics the ultrasonic properties of mammalian brain. The system concept was validated through the observation of shear wave displacements and calculation of their propagation speed from measurements of delay as a function of position.

2. Forcing Field Simulation Analytical and finite element model-based simulations were developed to assess the efficacy of alternative forcing field shapes for application in the *in vivo* system. These simulations are being used to guide the design of the next iteration of remote elastic wave generation transducer.

3. Brain Attenuation Measurements A formalin fixed *tursiops truncatus* brain was obtained on loan from Dr. Sam Ridgway. Non-invasive measurements of ultrasonic attenuation were made for several locations in the brain as a precursor to elastography measurements.

4. Hemorrhage and Edema Simulation A finite element model-based simulation effort was initiated for the purpose of assessing the ability of the *in vivo* system technology to detect the presence of hemorrhage and edema. Two potential approaches have been identified and are being modeled. In the first approach, the NIVMS system would be used to detect the effects of fluid inclusions on the propagation of shear waves in otherwise continuous elastic tissues. The second approach would use NIVMS to detect differences in local vibration amplitude, which occur due to discontinuities in density, when the entire head undergoes low amplitude, low frequency oscillation.

RESULTS

1. Ultrasonic Vibrometer Development Several efforts were carried out in FY09 to further refine and improve the performance of the ultrasonic vibrometer used to measure tissue displacements in the *in vivo* system. The salient results are described below.

- a. The most significant improvement to the system hardware was the addition of a 16-bit high-frequency polynomial waveform generator into the system (Tabor 2572A). This has permitted a variety different ultrasonic time waveforms to be used as carrier signals without sacrificing their required spectral purity. Work performed with the waveform generator revealed that the accuracy of the low-frequency sample timing depended on other features of the ultrasonic carrier signal and, in particular, on the location and temporal width of the signal pulse within a period. This revealed a design tradeoff in that the merits of a particular time waveform depend on the magnitude of the timing errors inherent in its use and on the nature of restrictions on the transmitted ultrasonic signal level.
- b. Another restriction on the system was imposed by the dynamic range of the analog-to-digital converter (ADC) that was used to record ultrasonic backscatter. The low end of the dynamic range is determined by the bit noise floor of the ADC, while the upper end of the dynamic range was set by the cross talk between transmitting and receiving rings of the NIVMS transducer. Since no other existing ADC card could be identified with specifications that offered a significant improvement, an alternative was investigated wherein the cross talk was suppressed by combining the receive ring output with a cancellation signal in a differential amplifier. The differential output, with the crosstalk largely removed, was connected to the ADC input. This scheme proved to be effective in making more efficient use of the ADC dynamic range. The significance of these results is that smaller signals could be measured with existing ultrasonic drive signal levels, or alternately that system drive signal levels could be lowered without adversely impacting system sensitivity.
- c. Several improvements have been made to the post-processing algorithm over the past year. A new time-domain formulation of the basic principles of system operation is the foundation of most of these improvements. The speed of the algorithm has been improved by piece-wise processing of the data stream in increments of one carrier period. In its current form, the processing algorithm can be run on a desktop PC in less time than was needed for the acquisition of the input data. Pseudo real-time processing (output with a constant delay) is therefore possible.

2. Forcing Field Simulations Analytical and finite element model-based simulations were developed to assess the efficacy of alternative forcing field shapes for application in the *in vivo* system. Specifically, comparisons were made between concentrated (point-like) force profiles, as are

commonly used in ultrasound elastography, and ring-shaped force profiles. The critical findings from the simulation effort are that:

- a. Ring-shaped force profiles may produce tissue displacements at the center of the forcing region which are the same size as those produced by concentrated force profiles, without any increase in risk of tissue damage. Moreover, ring force distributions produce a spatial displacement distribution that varies slowly inside the ring for much of the frequency range of interest. This result relaxes the requirement for the narrowness of the NIVMS transducer beamwidth.
- b. Ring-shaped force profiles produce significantly lower temperature increases in tissues, due to the distributed deposition of absorbed ultrasonic energy. Simulations of ultrasound-induced tissue heating were carried out using thermal properties of human brain, and showed that distributing the incident power in a ring geometry is particularly effective in tissues that are heavily perfused, even for ring radii of a few millimeters.

A companion numerical study was performed to investigate methods for generating ring-like force distributions with ultrasound transducers. Field intensities in absorbing media were calculated for several candidate transducer configurations. Of these, two configurations were identified as most promising, although with differing levels of complexity (number of independent channels), flexibility (ability to move the focal volume), and purity of beam shape. This study is expected to conclude by the end of the calendar year, resulting in a prototype design which will be developed and tested in 2010.

Overall, the results of the simulation studies have confirmed and quantified the degree to which ring-shaped forcing beams are both desirable and feasible to implement.

3. Brain Attenuation Measurements A formalin fixed *tursiops truncatus* head, with brain intact, was obtained on loan from Dr. Sam Ridgway for the purpose of system testing with real tissues. Prior to attempting elastographic measurements, the ultrasonic attenuation at several sites within the brain was measured non-invasively. Attenuation is a critical parameter for the *in vivo* system, as it directly impacts force generation levels, heat generation rates, and maximum working depth. Measured values of attenuation in the 2 MHz range were found to generally be lower than published values for human brain^{iv,v}. Considering possible impacts due to post-mortem handling and long-term formalin fixing, it is uncertain whether the measured values could be considered representative of living brain tissue. If the observed values are representative, they would imply that lower drive levels could be used for both the force generation and displacement measurement systems, further improving system safety.

4. Hemorrhage and Edema Simulation The primary results of this effort are those of the literature search used to define the range of parameters to be examined in the finite element model study. Information gleaned from general physiology^{vi,vii}, and marine mammal stranding^{viii,ix,x} literature has provided ranges of locations and characteristic dimensions of hemorrhage sites, as well as ranges of constituent fluid properties as a function time.

IMPACT/APPLICATIONS

There is considerable interest in the development of structural acoustic models for the cetacean head for two main reasons: 1) to better understand biomechanics of sound reception and production in cetaceans, and 2) to understand and hopefully mitigate any harmful effects of man-made sound on their

health and behavior. The development and validity of these models is severely limited by an almost complete lack of knowledge of the mechanical properties of the constituent living tissue. There is thus considerable interest in being able to measure these properties *in vivo*. The techniques and instrumentation investigated here should also have biomedical diagnostic application, including non-invasive examinations of stranded animals.

RELATED PROJECTS

Peter Rogers attended the NOPP experiment conducted by Ken Foote at the Naval Surface Warfare Center - Carderock in February of 2009 as an observer/consultant. This experiment made measurements of the acoustic field excited by a nearby source within a dolphin carcass with an array of embedded hydrophones. We expect to participate in the next NOPP carcass experiment to test our apparatus on intact fresh tissue. We hope to provide the NOPP group with material parameters they can use to analyze their results.

Michael Gray attended diagnostic imaging procedures conducted on a deceased late term Cuvier's beaked whale (*Ziphius Cavirostrus*) fetus at the University of Georgia College of Veterinary Medicine in June, 2009. The whale was found inside its mother, which was discovered deceased and stranded on the Georgia coast in 2008. The whale was imaged with both CT and MRI systems. The scans provided useful information regarding tissue dimensional scales, but also showed that this specimen was not a good candidate for *in vivo* system testing due to the significant amount of entrained gasses in the head tissues, presumably as a result of decomposition.

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